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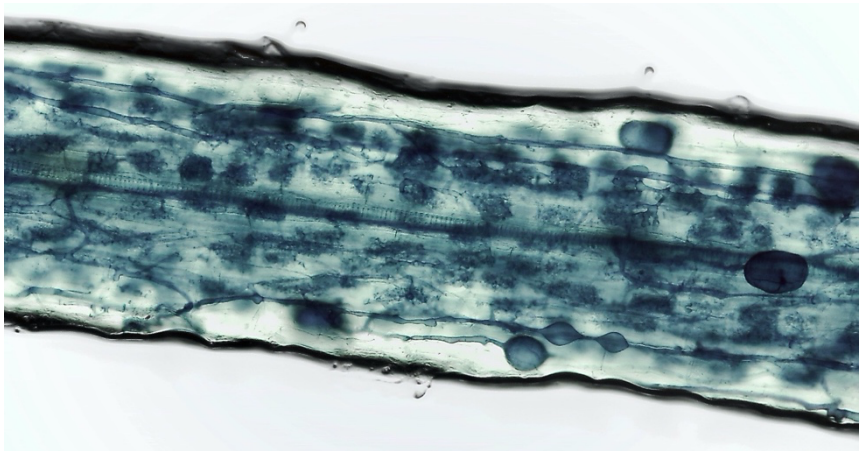
ZURICH UNIVERSITY OF APPLIED SCIENCES
DEPARTEMENT LIFE SCIENCES UND FACILITY MANAGEMENT
INSTITUT FOR ENVIRONMENTAL AND NATURAL RESSOURCES

Sustainable food production with biostimulants

Testing the effects of arbuscular mycorrhizal fungus *R. irregulare* and the bacteria *B. amyloliquefaciens* on plant growth and disease resistance



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Abstract

The soil beneath our feet holds a complex network of microorganisms that influence each other as well as the plants that grow in the soil. Promoting these soil communities offers new opportunities to create sustainable agricultural systems. Through their fungal network arbuscular mycorrhizal fungi (AMF) provide additional nutrients from the soil to plants. Hence, they represent a natural soil nutrient resource with the potential to reduce external fertilizer inputs in the future. The AMF *Rhizoglomus irregulare* and the bacterium *Bacillus amyloliquefaciens* are two biostimulants that associate with roots of many important agricultural crops.

In this study three field trials were carried out with the crops celery, celery root and root parsley, on three vegetable farms with organic and demeter management. The effect of the two biostimulants *R. irregulare* and *B. amyloliquefaciens* inoculated alone or in combination, on yield performance and disease suppression was evaluated by measuring the plant weight, colonization rate by the inoculated fungus in plant roots, plant nutrient concentrations and disease infection rates.

A positive effect of the inoculation with *R. irregulare* on plant growth was found in root parsley with a significant yield increase of 31 %. In celery and root parsley both inoculated and control plants showed high colonization levels, indicating that in these soils the occurrence of native AMF was already high. The crop celery root showed a significant increase in arbuscular root colonization. No significant positive effects of *B. amyloliquefaciens* on plant growth, as well as of both biostimulants on disease suppression were found.

The yield increase by *R. irregulare* inoculation in root parsley was not reflected in a higher colonization rate by AMF in the roots. However, analysis of the nutrient concentrations of the experimental plants shows, that inoculated plants presented significantly higher uptakes of N, P, C, Mg and Ca. It is assumed that the inoculated AM-fungus has replaced the native AM-fungi in the root. In the future, molecular methods present promising tools to investigate the arbuscular mycorrhizal root colonization at species level and therefore, to better understand the influence of inoculated AM- fungi.

Zusammenfassung

Der Boden unter unseren Füßen enthält ein komplexes Netzwerk von Mikroorganismen, die sich gegenseitig und gleichzeitig auch die im Boden wachsenden Pflanzen beeinflussen. Die Förderung dieser Bodengemeinschaften eröffnet neue Möglichkeiten, nachhaltige landwirtschaftliche Systeme zu gestalten. Durch ihr Pilznetzwerk liefern arbuskuläre Mykorrhizapilze (AMF) zusätzliche Nährstoffe aus dem Boden an Pflanzen. Folglich stellen sie eine natürliche Nährstoffressource im Boden dar, die das Potenzial hat, in Zukunft externe Düngereinträge zu vermindern. Der AMF *Rhizoglomus irregulare* und das Bakterium *Bacillus amyloliquefaciens* sind Biostimulanzien, die mit den Wurzeln einer Vielzahl von Kulturpflanzen assoziieren.

In der vorliegenden Studie wurden drei Feldversuche mit den Kulturen Stangensellerie, Knollensellerie und Wurzelpetersilie auf drei Gemüsebetrieben mit biologischer und demeter Bewirtschaftung durchgeführt. Untersucht wurde der Einfluss der beiden Biostimulanzien *R. irregulare* und *B. amyloliquefaciens*, allein oder in Kombination inokuliert, auf die Ertragsleistung und die Krankheitsunterdrückung. Der Einfluss durch die zwei Biostimulanzien wurde mit Messungen des Pflanzengewichts, der Kolonisierung der Wurzeln durch den inokulierten Pilz, der Pflanzennährstoffkonzentrationen und des Krankheitsschweregrades bestimmt.

Ein positiver Effekt der Inokulation mit *R. irregulare* auf das Pflanzenwachstum wurde in Wurzelpetersilie mit einer signifikanten Ertragssteigerung von 31 % festgestellt. Die Kulturen Stangensellerie und Wurzelpetersilie zeigten sowohl in inokulierten als auch in Kontrollpflanzen eine hohe Kolonisierung der Wurzeln durch AMF auf, was darauf hindeutet, dass in diesen Böden das Vorkommen von einheimischen AMF bereits hoch war. In Knollensellerie wurde ein signifikanter Anstieg in der Kolonisierung durch arbuskeln der Wurzeln beobachtet. Es wurde kein signifikanten Einfluss von *B. amyloliquefaciens* auf das Pflanzenwachstum sowie von beiden Biostimulanzien auf die Pflanzengesundheit festgestellt.

Die Ertragssteigerung durch die Beimpfung mit *R. irregulare* in Wurzelpetersilie widerspiegelte sich nicht in einer höheren Kolonisierung der Wurzeln durch AMF. Die Analyse der Nährstoffkonzentrationen der Versuchspflanzen zeigt jedoch, dass inokulierte Pflanzen signifikant höhere Aufnahmen von N, P, C, Mg und Ca aufwiesen. Es wird vermutet, dass der inokulierte AM-Pilz die einheimischen AM-Pilze in der Wurzel ersetzt hat. In Zukunft stellen molekulare Methoden vielversprechende Werkzeuge dar, um die Besiedlung der Wurzel durch AMF auf Artniveau zu untersuchen und somit den Einfluss inokulierter AM-Pilze besser zu verstehen.

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Abbreviations

AF	Antagonistic fungi
AM	Arbuscular mycorrhiza
AMF	Arbuscular mycorrhizal fungi/ fungus
ANOVA	Analysis of variance
B. amyloliquefaciens	*Bacillus amyloliquefaciens*
FZB42	Strain of *B. amyloliquefaciens*
G. mosseae	*Glomus mosseae*
MGR	Mycorrhizal growth response
MHB	Mycorrhiza helper bacteria
MUR	Mycorrhizal uptake response
PGPB	Plant growth promoting bacteria
R. irregulare	*Rhizoglomus irregulare*
SAF22	Strain of *R. irregulare*
qPCR	Real time quantitative Polymerase Chain Reaction

1 Introduction

1.1 Soil life and agricultural sustainability

To achieve high yields, modern agricultural systems follow a strategy of intensive land use, characterized by high inputs of fertilizers and pesticides. Whilst rising demands for agricultural products will pressure farmers to further intensify their production of crops, negative impacts on the environment have to be minimized (Bommarco et al. 2013). Land-use intensification has a reducing impact on soil life biodiversity and species richness and complexity (Tsiafouli et al. 2015; Banerjee et al. 2019). On the contrary the promotion of internal regulatory ecosystem processes provided by biological soil communities, offers the opportunity to enhance sustainability of agricultural practices, while reducing external inputs (Bender et al. 2016; Zhang et al. 2020). Meanwhile, pressure from soil-borne pathogens increases in disturbed ecosystems, such as those found in today's modern agriculture, which often leads to large economic losses (Linderman 2000). Furthermore, soil-bound pathogens will be fueled by global warming, including diseases of important food crops. This may affect worldwide food security in the long term (Delgado-Baquerizo et al. 2020).

1.2 Use of biostimulants in vegetable production

The search for environmentally friendly alternatives has led researchers to focus on the biological control of fungal diseases using biostimulants, such as antagonistic fungi (AF) and other microorganisms (Adnan et al. 2019). In order to reduce fertilizer use, it is increasingly necessary to benefit from naturally occurring nutrient resources. Biostimulants have the potential to replace the use of chemical fertilizer by up to 75% and produce the same plant growth, yield and nutrient uptake as when treated with the full fertilizer rate (Adesemoye et al. 2009). In addition to their positive influence on nutrient uptake, they also play an important role in plant health. Their mode of action is complex and the cumulative result of various interactions between plant, pathogen, antagonists and environmental factors (Böhme et al. 2016). In this study, the role of AMF as well as the use of *B. amyloliquefaciens* in plant production is described, followed by an overview of research looking at the interactions between *R. irregulare* and *B. amyloliquefaciens*.

1.3 The role of AMF in agricultural systems

Arbuscular mycorrhizal fungi form a symbiosis with about 71% of all vascular plant species, including many important crops, and can be found in the majority of soil types and most agroecosystems (Oehl et al. 2003; Wang and Qiu 2006; Van der Heijden et al. 2015; Davison

et al. 2015). AMF are present within the plant root where they form mycorrhizal structures within and between the cells of plant roots (Fig.1) and also in form of mycelium in the soil (Smith and Read 2010). AM fungi form a widespread symbiotic interaction with plants leading to benefits for the plant in terms of growth, nutrition and protection (Bender et al. 2019; Bagy et al. 2019) Through intimate associations, AMF can provide up to 80% of the phosphorus needs of the host plant (Smith and Read 2010).

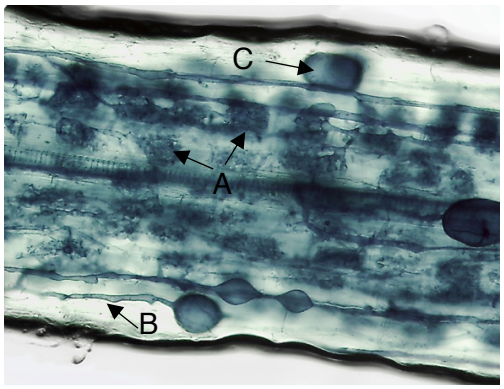

Figure 1: AM fungal structures within a root fragment of root parsley at 200- fold magnification. The letter A shows an arbuscule, where the nutrient exchange with the plant takes place. Letter B shows hyphae, the organ that allows the fungi proliferation inside the plant and in the soil. The letter C represents a vesicle, a storage organ of the fungi. Anne-Miamed Fehr

AMF have also been shown to reduce nutrient losses caused by leaching and also reduce N_2O emissions from soil (Bender et al. 2014; Cavagnaro et al. 2015; Storer et al. 2018). Furthermore, AMF may contribute to enhance plant resistance to environmental stress and enhance tolerance or resistance to root pathogens (Borowicz 2001). In consequence, the effects of AMF on plant health and productivity are receiving increasing attention.

The promotion of AMF in the soil can play an important role for sustainable agricultural systems with low additions of nutrients, such as organic and demeter management systems. Possibly together with mycorrhiza helper bacteria (MHB), they can provide nutrients for crop plants (Johansson et al. 2004). In todays vegetable production AM fungi are faced with different types of disturbances due to different agricultural management strategies. For example, tillage and milling are common soil management practices in vegetable production. These soil processing techniques cause a physical disruption of fungal mycelia and can also influence AMF by changing physicochemical properties of the soil (Säle et al. 2015). Crop rotation may cause a temporal absence of host plants for AM fungi (Harinikumar and Bagyaraj 1988). Fertilization and application of pesticides represent chemical disturbances, and their impact on AM fungi depend strongly upon application rates and type of product (Helander et al. 2018).

Recent research of Bender et al. (2019) has shown, that inoculation of AMF in soils with low native AMF abundance could present a strategy to promote AMF in the soil, and therefore increase plant growth. AMF inoculation success and AM fungal persistence in soils are

determined by three factors. First of all, the introduced AMF species must be able to thrive under the imposed circumstances. Also, the field carrying capacity plays an important role, as there must be a habitat niche available to newly introduced AMF. At last, the priority effects determine the influence of timing and competition on the establishment by native AMF communities (Verbruggen et al. 2013; Niwa et al. 2018).

There are many instances that show the influence of AMF on plant growth. However there are still few investigations concerning inoculation and management to increase AMF colonization as part of a commercial agricultural practice (Smith and Read 2010). An effective inoculant represents the AM fungi *R. irregulare*. With a worldwide distribution *R. irregulare* is a common AM fungi, and is present in a wide range of ecosystems, including many agricultural fields in Switzerland (Öpik et al. 2006; Oehl et al. 2010). Several studies reported that AMF *R. irregulare* strain SAF22 has the highest establishment potential compared to other AMF tested (Imperiali et al. 2017).

1.4 Use of *Bacillus amyloliquefaciens* in plant production

A range of root-associated *Bacillus* species has already been developed as biostimulant agents due to their contribution to plant protection (Andrić et al. 2020). The positive effects on plant growth and disease suppression by *B. amyloliquefaciens* strain FZB42 have been tested in numerous greenhouse and field trials on a variety of crops, including tomato, cucumber, tobacco and lettuce (Gül et al. 2008; Wang et al. 2009; Chowdhury et al. 2013). Fan et al. (2018) has demonstrated the ability of *B. amyloliquefaciens* to colonize roots, by using a CFP-labelled FZB42 strain in maize, tomatoes, lettuce, *Arabidopsis thaliana* and *Lemna minor*.

In Switzerland as well as in many other countries, the Bacillus strain FZB42 is an approved bioagent in agriculture and is used to increase yields and against fungal and bacterial pathogens (Chowdhury et al. 2015). Bacteria of the genus *Bacillus* are particularly suitable for the production of microbial products, as their endospores can survive for long periods of time (Borriss 2016).

1.5 Interaction between AMF *R. irregulare* and *B. amyloliquefaciens*

It is likely that the long co-evolution of plants and AM fungi did not occur independently from the associated bacterial flora (Frey-Klett et al. 2007). The interaction of AMF with bacterial communities takes place in the rhizosphere as well as on its own hyphal network, commonly referred to as the mycorrhizosphere (Rambelli 1973; Linderman 2008). Some bacteria can have a direct influence on plant physiology, while others engage in a more indirect synergism

in the mycorrhizosphere and thus support plant growth (Azcón-Aguilar and Barea 1997; Bharadwaj et al. 2008). Direct synergism may be based on improved nutrient uptake by the plant with help of the bacteria, therefore they act as plant growth-promoting bacteria (PGPB)(Barea et al. 2002). Another mode of action lies in the stimulation of the spore germination and the growth rate of AM fungal hyphae, whereby the extent of AMF colonization is increased (Mayo et al. 1986). Such bacteria are called mycorrhiza helper bacteria (MHB) (Mayo et al. 1986; Barea et al. 2002; Frey-Klett et al. 2007; Bharadwaj et al. 2008; Nazir et al. 2010). As early as 1962, Mosse (1962) showed that some MHB are able to promote the AM fungal spore germination of the species *G. mosseae*. In some plant species, AMF colonization may even strongly depend on the presence of MHB bacteria (Frey-Klett et al. 2007; Xie et al. 2018). A meta-analysis by (Hoeksema et al. 2010) showed that the more complex the soil microbial community, the more positive is the response of plants to mycorrhizal inoculation.

Three types of interactions between MHB and AMF deserve special attention because of their practical importance in plant production and their potential applications in vegetable farming: nutrient mobilization from soil minerals, fixation of atmospheric nitrogen and protection of plants from root pathogens (Artursson et al. 2006). This study will focus on the mobilization of nutrients and protection against root pathogens.

Co-inoculation of *B. amyloliquefaciens* and AMF have resulted in positive effects on growth and yield parameters (Yusran et al. 2009; Mikiciuk et al. 2019) and a reduction in disease severity and frequency (Rashad et al. 2020). *B. amyloliquefaciens* is able to facilitate AMF colonization acting as a MHB when co-inoculated with AMF (Xie et al. 2018). Yusran et al. (2009) tested single and combined inoculations of AMF and FZB42. Results showed that the effect on dry matter and shoot nutrient concentrations in the combined treatment was increased in comparison with the single treatment with AMF (Yusran et al. 2009). These results indicate that selected MHB and AM fungi could be co-inoculated to improve the formation and performance of AMF-symbiosis with plants. Even though the combination of soil beneficial organisms may suggest positive effects when AMF inoculum is combined with the bacteria *B. amyloliquefaciens*, it remains to be further validated whether root colonization by *R. irregulare* is particularly facilitated and yield increased if applied in combination with the bacteria. The synergistic effects of bacteria and mycorrhizal fungi in terms of their combined beneficial effects on plants is currently a focus of scientific research. As early as 1997 (Linderman) showed that PGPR have a strong stimulatory impact on the growth of AM fungi. These results suggest that selected bacteria in the rhizosphere of plants and AMF could be co-inoculated to optimize the formation and functioning of the AMF symbiosis (Artursson et al. 2006).

1.6 Study overview

The objective of this study was to evaluate the influence of AMF (*R. irregulare* strain SAF22) inoculation, alone or in combination with *B. amyloliquefaciens*, on plant growth and on tolerance to disease of celery *(Apium graveolens var. dulce),* celery root *(Apium graveolens var. rapaceum)* and root parsley *(Petroselinum crispum subsp. Tuberosum).* In this regard three soils of farms with organic and demeter management systems from the north-east of Switzerland were investigated. The farms faced various problems with disease pressure in previous years. The results of this study will help to develop sustainable farming methods in vegetable production with the aim to reduce the use of fertilizers and pesticides.

It was hypothesized that improved root colonization by single or combined application of *R. irregulare* strain SAF22 and *Bacillus amyloliquefaciens* strain FZB42 will enhance plant growth and health of celery and root parsley. Furthermore, It was hypothesized that plants grown in soils from differently managed fields become differently colonized by the inoculated AM fungus. Specifically, the present study poses the questions whether (i) single and/or combined treatments with *R. irregulare* and *B. amyloliquefaciens* FZB42 produce higher yields compared to non-treated plants and whether (ii) the inoculation with *R. irregulare* and *B. amyloliquefaciens* FZB42 increases the resistance of plants against soil-borne fungal diseases which may occur naturally in the field.

2 Material and Methods

2.1 Location and time frame of the experiment

The field trials were carried out on two fields with organic management in Tägerwilen TG and one field with demeter management in Scherzingen (TG) in Switzerland (Table 1).

Table 1: Details on the characteristics of the three field experiments (E1, E2 and E3) used to assess the effect of inoculation of *R. irregulare* and *B. amyloliquefaciens* on growth, yield and health of celery and root parsley.

Field trials	Experiment 1	Experiment 2	Experiment 3
Coordinates SN/EW	2'728'731 / 1'280'350	2'728'372 / 1'279'740	2'733'550 / 1'277'728
Management system	Organic	Organic	Demeter
Plants	Celery	Root parsley	Celery root
Treatments	C: (no inoculants)	C: (no inoculants)	C: (no inoculants)
	B: Bacillus amyloliquefaciens	B: Bacillus amyloliquefaciens	B: Bacillus amyloliquefaciens
	M: AMF inoculum	M: AMF inoculum	M: AMF inoculum
	MC: AMF control inoculum	MC: AMF control inoculum	MC: AMF control inoculum
	BM: Bacillus and AMF inoculum	BM: Bacillus and AMF inoculum	BM: Bacillus and AMF inoculum
	BMC: Bacillus and AMF control inoculum	BMC: Bacillus and AMF control inoculum	BMC: Bacillus and AMF control inoculum
Sowing/planting date	01.04.20 and 15.04.20	08.05.20	27.05.20
Inoculation date AMF	22.04.20	08.05.20	27.05.20
Inoculation date Bacillus	20.05.20	12.06.20	12.06.20
Harvest date	19.06.20	14.09.20	06.10.20
Number of days inoculated	59	130	133
AMF inoculum in ml per plant	200	14	200
Bacterial inoculum concentration	2 ml RhizoVital42/2400 ml water/plot	1 ml RhizoVital42/1000 ml water/plot	2 ml RhizoVital42/2400 ml water/plot
Number of replicates per treatment	Six replicates in block design	Six replicates in randomized complete block design	Six replicates in randomized complete block design
Size of plots	One row 6m / 3m length	Two rows 6m / 3m length	One row 6m / 3m length
Distance between plants in cm	25-30	3.5	25
Number of plants harvested/ plot	5	all plants along one meter (~28)	varied (4-6)

2.2 Biostimulants

The AMF *R. irregulare* strain SAF22 was used as an inoculant in this experiment. For the treatment with *B. amyloliquefaciens* the product RhizoVital 24 from Andermatt Biocontrol was chosen which contains a minimum of $2{,}5\times10^{10}$ spores/ml. In all three field trials, the same treatments were applied with the two biostimulants inoculated alone or in combination. In total, the four treatments AMF inoculum (M), AMF control inoculum (MC) Bacillus and AMF inoculum (BM) and Bacillus with AMF control inoculum (BMC) were applied. In addition, the treatment Bacillus (B) was applied directly on the field without the inoculum substrate and a control treatment (C) was included with no inoculum at all.

2.3 Experimental design

For the experimental design, each of the six treatments was assigned a plot of 3 meters in length. Treatments B and C were applied to plots of 6 meters in length. Each treatment was replicated six times, resulting in a total of 36 plots (Fig. 2 and appendix A.1, A.2).

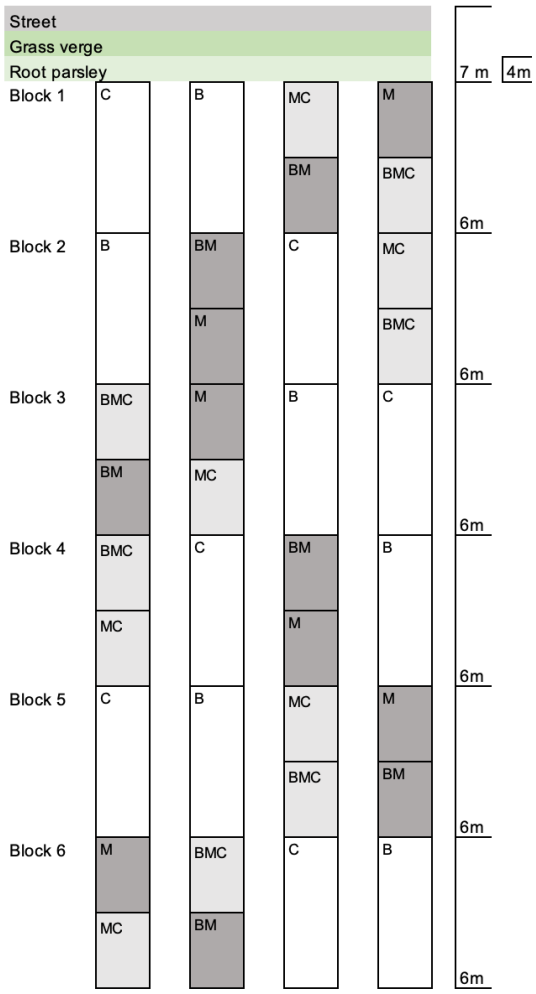

Figure 2: The field trial in root parsley with its completely randomized block design is shown. For each treatment six replicates were performed. Each of the six blocks contain the six treatments.

2.4 Inoculum production

The inoculum of the fungus *R. irregulare* strain SAF 22, was propagated in the greenhouse with *Plantago lanceolata* as a host plant. 5 L pots were filled with autoclaved 3:17 sand-soil mixture and inoculated with 5% inoculum. During cultivation the pots were watered regularly. Every second week, 20 ml of a modified Hoagland solution (Hoagland and Arnon 1950), containing one quarter of the original P concentration, was applied per pot. After 3 months of growth, pots were left to dry out, and aboveground biomass was discarded.

The roots were then cut into pieces smaller than 5 cm and mixed thoroughly with the rest of the substrate to serve as the soil inoculum. A non-mycorrhizal control was prepared following the same protocol, but without adding the fungus. This mock treatment was termed AMF control. The *R. irregulare* inoculum contained roots that were at least 82 % colonized. No root colonization by AMF was observed in the control inoculum. Root colonization by *R. irregulare* was assessed by the same method as described in chapter 2.9.

2.5 Inoculation of the biostimulants

In the first experiment (E1), seedlings of celery had already been planted 3 weeks before the time of inoculation. The planted seedlings were carefully removed from the soil. 200 g of AMF inoculum and control inoculum were then applied per plant to a depth of 20 cm to the soil (Fig. 3). In the experiment with celery root (E3), the seedlings were inoculated at the day of planting. The procedure was the same as for E1. The planting distance in E1 and E3 was 30 cm between and 25 cm within the rows.

As root parsley is cultivated in the field by direct sowing, in the experiment with root parsley (E2) the AMF inoculum was applied in the soil directly before sowing. With a shovel the soil was removed from the dams forming a 10 cm deep furrow. A meter was placed next to the furrow and 200 ml / 25 cm inoculum was applied resulting in 4 x 200 ml inoculum per 1 meter. The AMF Inoculum was applied manually with 400 ml glass beakers. The farmer then sowed directly onto the inoculated plots in two rows with a sowing distance of 3.5 cm (Fig. 3).

The treatment with *B. amyloliquefaciens* was carried out approximately one month (E1 and E2) and two weeks (E3) after the inoculation with *R. irregulare*. For each treated plot in E1 and E3, 2 mL of the product RhizoVital 24 from Andermatt Biocontrol was mixed with 2400 mL water. The product/water ratio in E2 was 1mL RhizoVital 24 mixed with 1000 mL water. The solution was then applied on the experimental plots with a watering can.

Figure 3: The celery seedlings were removed from the soil and the inoculum applied beneath the plant (A). In root parsley the inoculum was applied beforehand (B-C) and plants were sown directly into the inoculum (D). Anne-Miamed Fehr

2.6 Sampling

Prior to inoculation, two soil samples were taken at a depth of 0-20 cm with an edelmann soil-auger (diameter: 2.5 cm, length 20 cm) from each of the 36 plots. The soil samples were then combined to form a mixed sample per block. Soil properties are given in table 2.

To investigate root colonization by AMF in E1, soil samples were collected from each plot on the day of harvest. Four soil samples were collected in proximity to plants. The samples were then pooled to form a composite sample per plot. Roots were either washed from the soil (E1) or directly cut from the roots (E2 and E3). Roots were then cut into pieces of 1-2 cm. For the assessment of root colonization one subsample was stored in 50% ethanol. Another subsample was frozen by -60° for later DNA extraction. In all three experiments, weed pressure was relatively low at the time of root collection. Therefore, it is assumed that the roots from the soil samples are similarly representative as those collected directly from the root.

2.7 Harvest and processing

The plants were harvested shortly before the farmers' harvest. The number of plants harvested differed between the three experiments due to the varying number of plants per plot. Subsequently, in E1 five plants, in E2 all plants along one meter and in E3 5 or 4 plants were harvested from the center of the plots. Due to high yield loss caused by significant disease infestation, only 5 or 4 plants were harvested in E3. In this case average plant weight per plot was calculated. The roots of the plants were then cut from the sprout in order to collect the sprouts and roots separately for biomass and nutrient analysis. In E1 only the sprout was harvested. The sprouts and roots were weighed separately fresh and then dried at 60° for 48 hours for nutrient analysis. A subsample was dried at 105°C for 30 hours to obtain the dry weight. For the measurement of nutrient concentration, the plants were ground in a cutting mill.

Table 2: Selected soil properties of the three field sites investigated.

Field site	Organic C (mass%)	Humus (mass %)	pH	Total N (mass %)	Total soil P (mg/kg soil)	Mg (mg/kg soil)	K (mg/kg soil)	Sand (mass %)	Clay (mass %)	Silt (mass %)
E1	1.98	3.41	7.64	0.27	27.95	6.9	1.82	59.5	14.3	23.05
E2	2.12	3.64	7.46	0.32	7.55	11.4	1.07	57.5	19.55	19.5
E3	3.16	5.44	7.34	0.4	39.3	12.25	7.63	39.2	23.55	31.85

2.8 Assessment of diseases

In E2 and E3, diseases occurred towards the end of the cultures. In E2, the average disease incidence per plot was surveyed using a scoring system that recorded the percentage of infestation. In E3, scoring was done two weeks before harvest and on the day of harvest. The scoring system included five points ranging from 1 = no infestation to 5 = all leaves infested. Since a difference in plant growth height was observed, the average plant height per plot was also recorded in E3.

2.9 Assessment of AMF root colonization

To assess root colonization by AM fungus *R. irregulare* the roots were cleared with KOH and stained with an ink-vinegar solution (Vierheilig et al. 1998). For each replicate, approximately 30 root fragments were prepared on a microscope slide. The AM fungal root colonization was quantified by a modified line-intersection method for 100 intersections at magnification x 200 (McGONIGLE et al. 1990). Establishment success of the inoculated fungus was assessed by comparing root colonization parameters The establishment success of the inoculated fungus was defined as the percentage increase of *R. irregulare* strain SAF22 in root length colonization. The Abundance of AM fungal structures in roots of celery and root parsley either inoculated with C, B, M, BM, MC, BMC was investigated by assessing the following parameters: Total, vesicular, arbuscular and hyphal root colonization.

2.10 Nutrient analysis

The nutrient analyses of the soil and plants were performed by the laboratory of the Environmental Analysis Research Group at Agroscope, Reckenholz, Switzerland using their reference methods (FAL et al. 1996). The principles of each method are described below.

Soil physical and chemical properties (Table 2), were analyzed by extracting the readily soluble and immediately plant-available components of Ca, Mg, K, and P with water from air-dried soil. The extracts were then measured for Ca, Mg, and K concentrations by atomic absorption spectrophotometry. Available P contents were measured with the CO_2 method. Total C and total N contents were determined using the Dumas burning method (ISO/TC 34 Food products 2018). When analyzing the nutrient contents of the plants, the samples were first converted to crude ash after oxidative heat treatment until constant weight was reached. The crude ash was then mixed with HCl 6 M and filtered. Inductively coupled plasma optical emission spectroscopy was used to determine the nutrient content from the filtrate. Elemental analysis of the dry, ground plant material was performed to determine the N content (FAL et al. 1996).

2.11 Mycorrhizal growth response

Mycorrhizal growth response (MGR) was calculated as described in (Köhl et al. 2016) as a measure for the percentage change in plant biomass in plots inoculated with the AM fungus relative to mean biomass of control plots. Likewise, mycorrhizal nutrient uptake (MUR) of the plant was calculated based on the percentage change in nutrient content of the plots inoculated with *R. irregulare* relative to the mean of the control plots (Table 3 in appendix B.1).

2.12 Statistical analysis

Data were managed using Microsoft Excel 2020 for Mac. For statistical analysis, the software R Studio version 1.3.959 was used (R Core Team 2020). The significance level for all tests was set at alpha = 5%. Normal distribution was checked using QQ-Plots (visual analysis), as well as Kolmogorov-Smirnoff and Shapiro-Wilk tests. Comparisons of the different treatments were performed using two-way ANOVAs if the normal distribution of the residuals was given.

The package multcomp was used for multiple comparisons analysis (Hothorn et al. 2008). In case of significant ANOVA results post hoc analysis was performed using pairwise Tukey tests. Results of post hoc analysis were translated into letters. Different letters show significant mean differences ($p < 0.05$) according to Tukey test.

The package lme4 was used to perform a linear mixed effects analysis of the relationship between the different parameters and treatment (Bates et al. 2015). As fixed effects, two factors AMF and Bacillus (with interaction term) were entered into the model. As random effects, the intercept for block was given.

The package sciplot was used for data visualizations (Morales and Murdoch 2020). Results were visualized using bargraphs. Correlations between biomass and root colonization were performed using the Pearson correlation coefficient if data was normally distributed. For not normally distributed variables the Spearman rank correlation coefficient was used.

Significant differences of the mycorrhizal growth response (MGR) and mycorrhizal uptake response (MUR) from control mean values were assessed by one sided t-tests.

The effects of AMF inoculation alone or in combination with Bacillus were analyzed using only the data of treatments M, MC, BM, BM and BMC. In E2, the total dry and fresh biomass of the harvested plants within 1 meter was evaluated. On the other hand, for each plot the weight per plant was calculated and statistically evaluated (Fig 18. Appendix B.2). In E1 and E3 statistical analysis was performed with data including all plants harvested per plot.

3 Results

In this chapter, the influence of *R. irregulare* and *B. amyloliquefaciens* applied alone or in combination on plant growth is shown. Furthermore, the establishment of *R. irregulare* in the root is shown, and how root colonization rates differ between treatments. Effects on nutrient uptake are presented with the mycorrhizal nutrient uptake response. At last, the effect of *R. irregulare* and *B. amyloliquefaciens* on plant health is presented in this chapter. The focus of this paper will be on the results from the field trial E2 in root parsley. The trials E1 in celery and E3 in celery root are not presented in depth, as the results lose their significance due to the late inoculation in E1 and the loss of a quarter of the trial area in E3.

3.1 Effects on plant yield

In root parsley, the total dry and fresh biomass of the harvested plants within 1 meter was evaluated. With a yield increase of 31 %, root dry weight was significantly higher (p= 0.03) in the treatment with *R. irregulare* (M) compared to the control treatment (MC) (Fig. 4). On the other hand, for each plot the weight per plant was calculated and statistically evaluated, but no significant results were found here (Fig. 18 in appendix B.2). In root parsley no effect of inoculation on shoot fresh and dry weight was observed (p=0.487, p=0.32) (results not visualized).

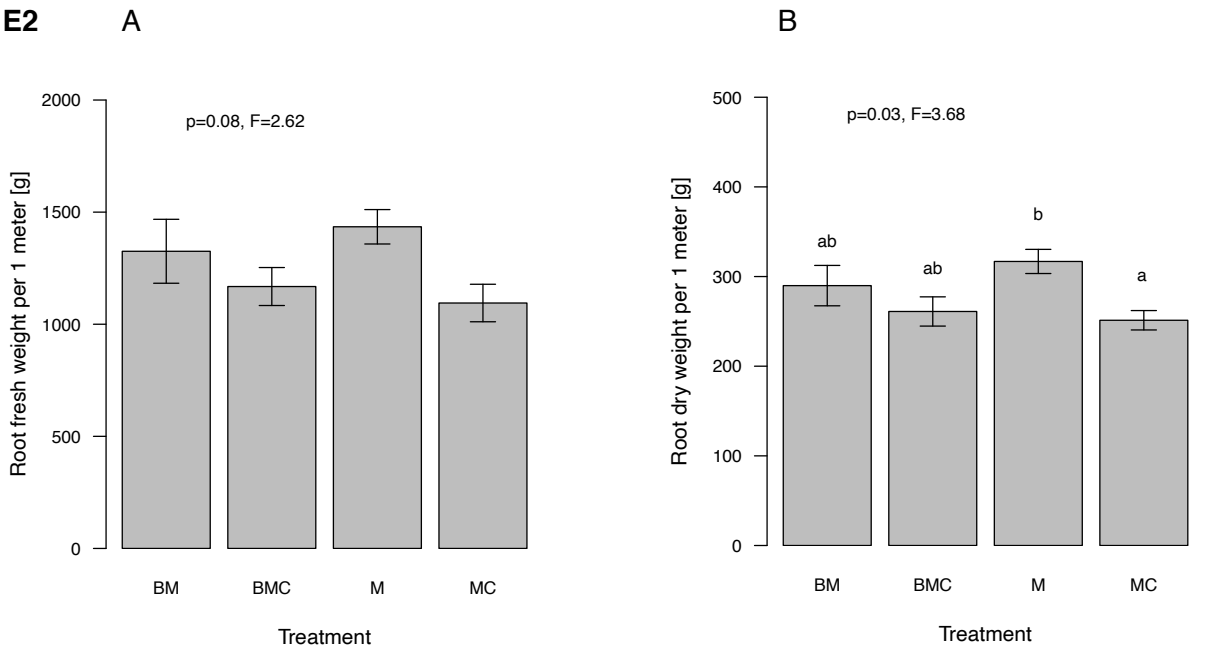

Figure 4: Effects of inoculation by the treatments BM, BMC, M and control (MC) in root parsley (E2) on (A) produce fresh and (B) produce dry weight. ANOVA results for the effects of Inoculation on fresh and dry produce weight are given. In (A) a tendency of higher yield of (M) in comparison with the control (MC) can be observed (p= 0.080, F= 2.62). In (B) a significant increase in dry weight with treatment M was found (p=0.030, F=3.68). Different letters show significant mean differences (p<0.05) according to Tukey test. Bars represent means of 6 replicated plots per treatment +/- 1 SE.

By calculating the mycorrhizal growth response (MGR), the effect of AM fungal inoculation on root parsley biomass was measured compared to control treatments. In root parsley a significant growth response of inoculated plants with a 23% change in root fresh biomass was found, compared to control plants (Fig. 5). Both fresh and dry root biomass differed significantly from the control treatments (p= 0.007, p= 0.001).

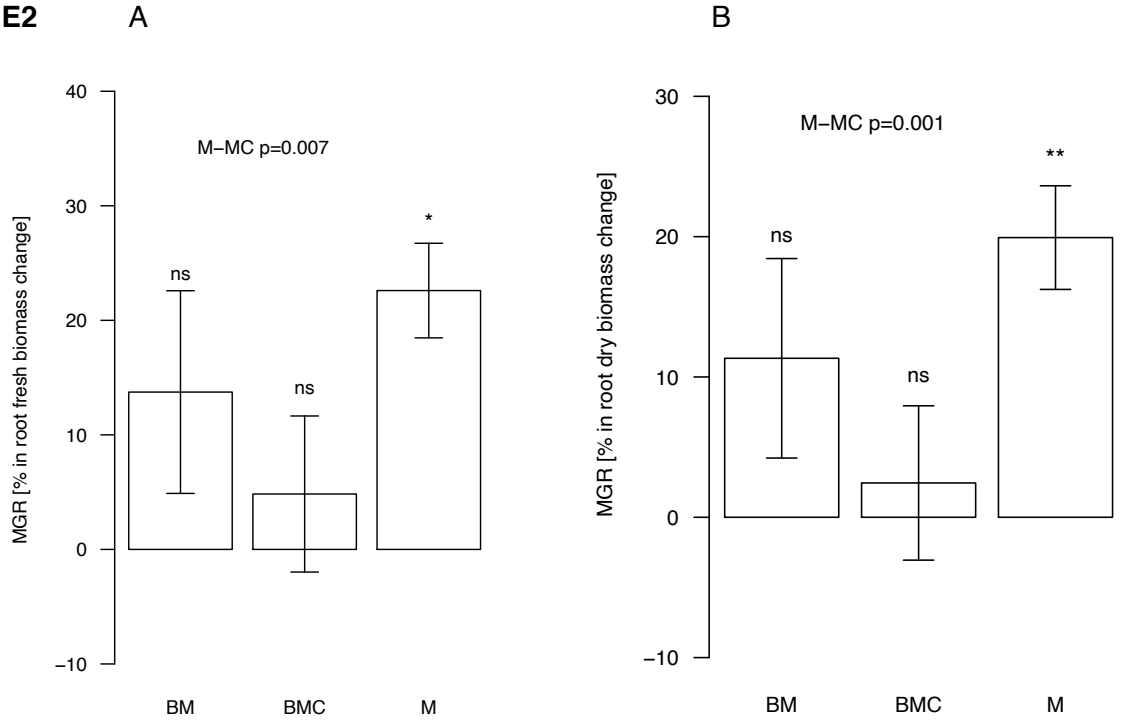

Figure 5: MGR of treatments M, BM and BMC in(A) fresh and (B) dry root weight of root parsley (p=0.097 and p= 0.18). Significant differences from the mean value of control treatment was assessed by one-sided t-tests. Test results are given above bars as ns > 0.1, * $p < 0.05$ and ** $p < 0.001$. Bars represent means of 6 replicated plots per treatment (five replicated plots for treatment BM) +/- 1 SE.

Similarly, in the two trials with celery (E1) and celery root (E3), a tendency towards higher yields was observed in the plants inoculated with AMF compared to control plots, with a yield increase in fresh produce of 5% in E1 and 2% in E3 (Fig. 6 and 7). However, no significant effect of AMF on Biomass of celery and celery root was found in these trials.

Furthermore no significant effect of the single application of *B. amyloliquefaciens* (BMC) or combined treatment (BM) on plant biomass could be detected in any field trial. The median of BM and BMC were found to be below the median of M in all trials.

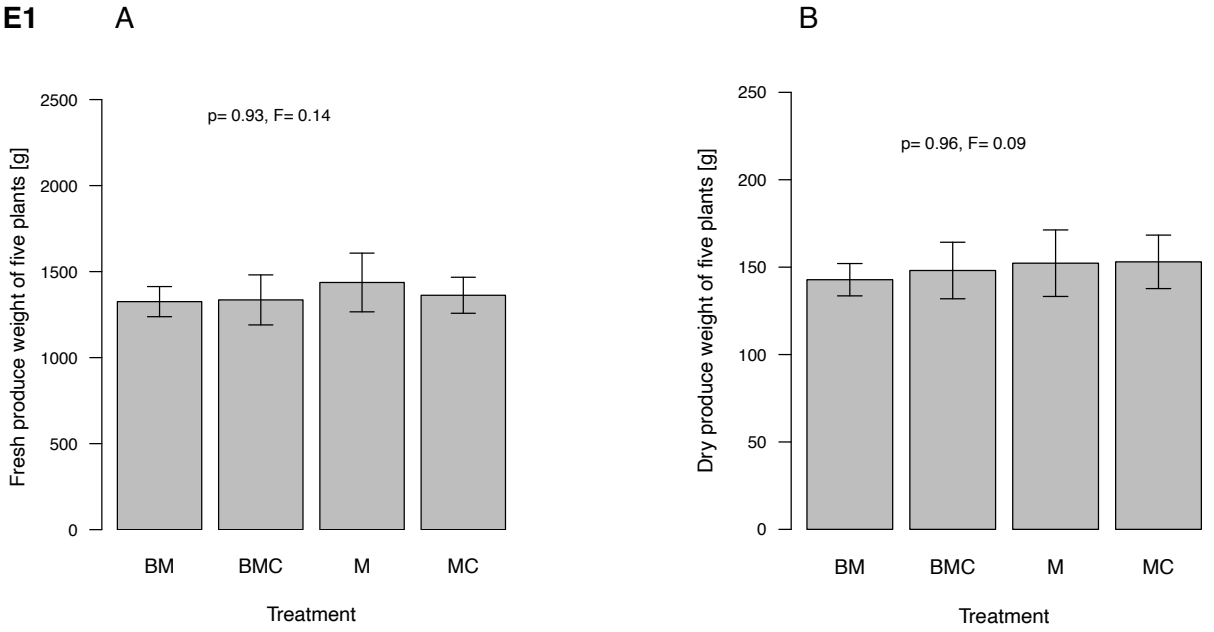

Figure 6: In celery no effects of inoculation by the treatments BM, BMC, M compared to control treatments on (A) fresh produce weight (p= 0.93) and (B) dry produce weight (p= 0.96) were found. Bars represent means of 6 replicated plots per treatment +/- 1 SE.

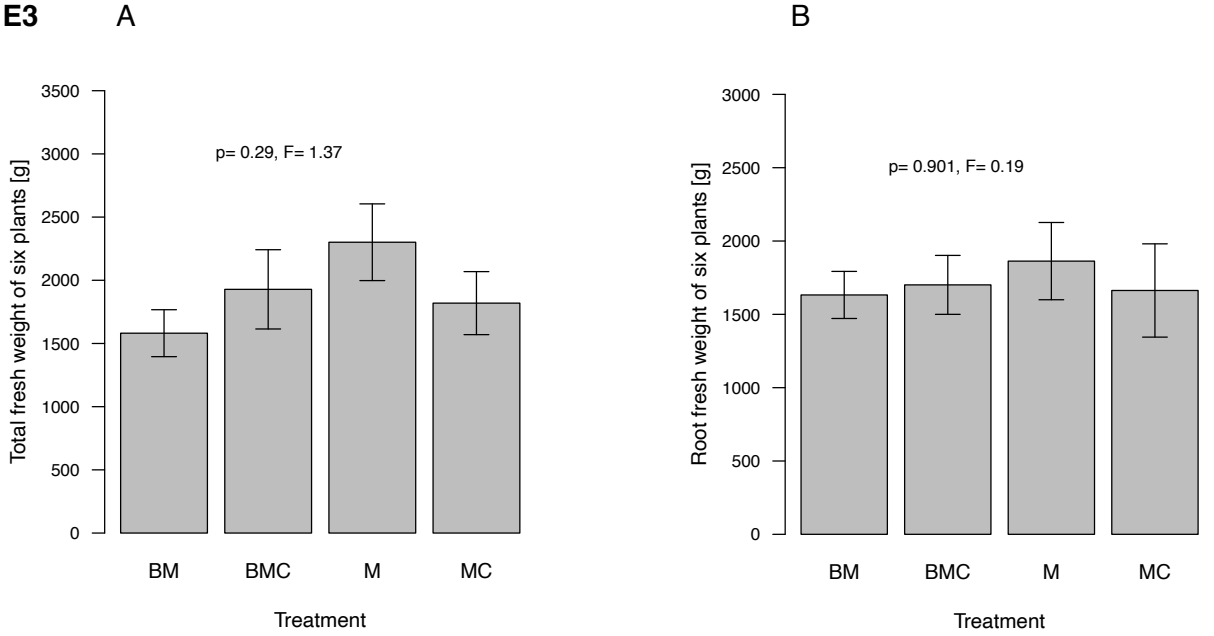

Figure 7: Results for Biomass in celery root show a tendency of yield increase by treatment with *R. irregulare* in all measured parameters, but no significant differences between treatments (p- values from 0.29 to 0.9). Bars represent means of 4/5 replicated plots per treatment +/- 1 SE.

3.2 Root colonization by AMF

Establishment success of the inoculated fungus was assessed by comparing root colonization parameters including (A) total, (B) vesicular and (C) arbuscular colonization. Inoculation with *R. irregulare* and *B. amyloliquefaciens* affected total AM fungal root colonization differently across sites. A tendency towards higher total root colonization in plants treated with AMF is visible in all field trials but no significant effects were observed in all experiments. However total root colonization levels were already high in control plots of E1 and E2. Average root length colonized in control plots (MC) show mean total root colonization levels of 59% in E1, 58% in E2 and 37% in E3 (Fig. 8A, 9A and 10A).

Effects of inoculation on arbuscular root colonization also differed across sites. Significant effects by inoculation with *R. irregulare* on arbuscular root colonization levels were detected in celery root (E3). The percentage of arbuscular root colonization was significantly higher in celery root plants treated with *R. irregulare* (M) than in control plants (MC) (TukeyHSD p= 0.015)(Fig. 10). Average root length colonized by arbuscules was 48 % in inoculated celery root plants, while it was 25% in control plants. This corresponds to an increase of 23% in arbuscular root colonization.

E1 Celery

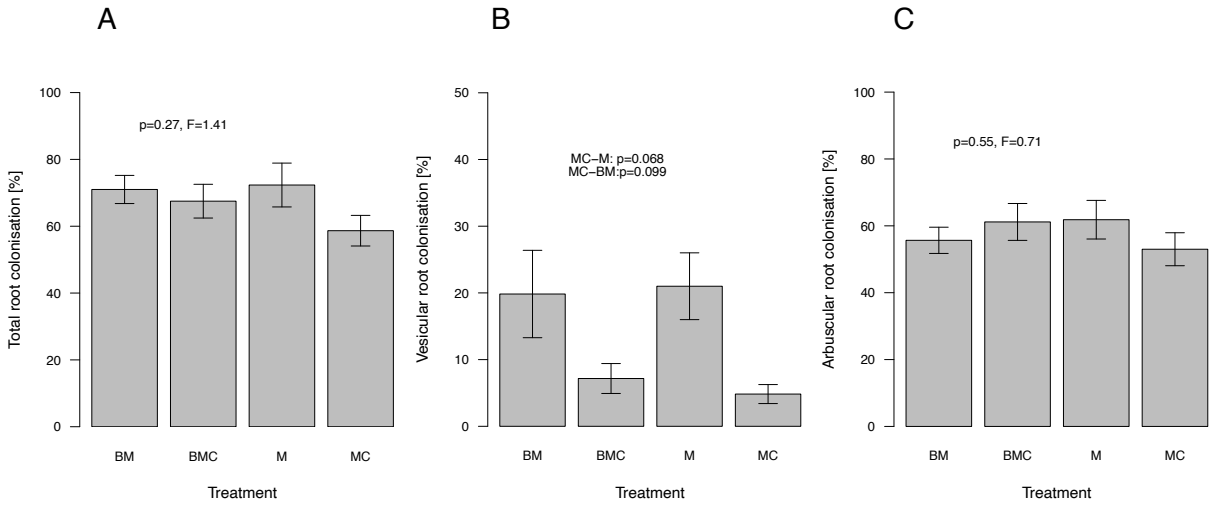

Figure 8: In celery, Versicular root colonization (B) is tending to show higher coloniszation levels in treatment M than in control MC (p= 0.068) and also in treatment BM compared to the control MC (p=0.099). No significant diffrences were found in (A) total (B) vesicular and (C) arbuscular root colonisation levels. Bars represent means of 6 replicated plots per treatment +/- 1 SE.

E2 Root parsley

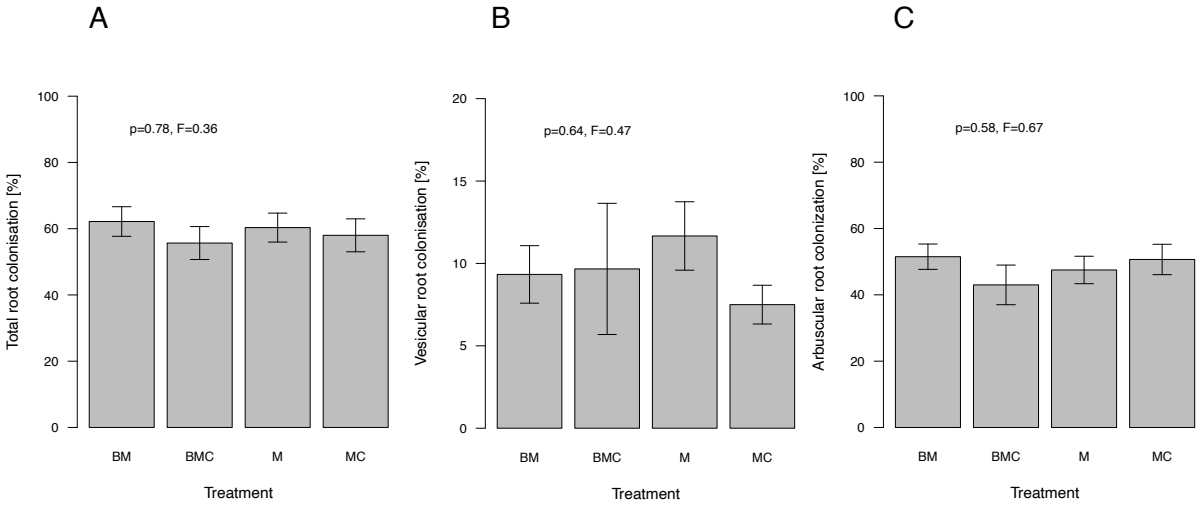

Figure 9: In root parsley no significant effects of treatments M, BM and BMC on (A) total, (B) vesicular and (C) arbuscular root colonization levels were observed. Bars represent means of 6 replicated plots per treatment +/- 1 SE.

E3 Celery root

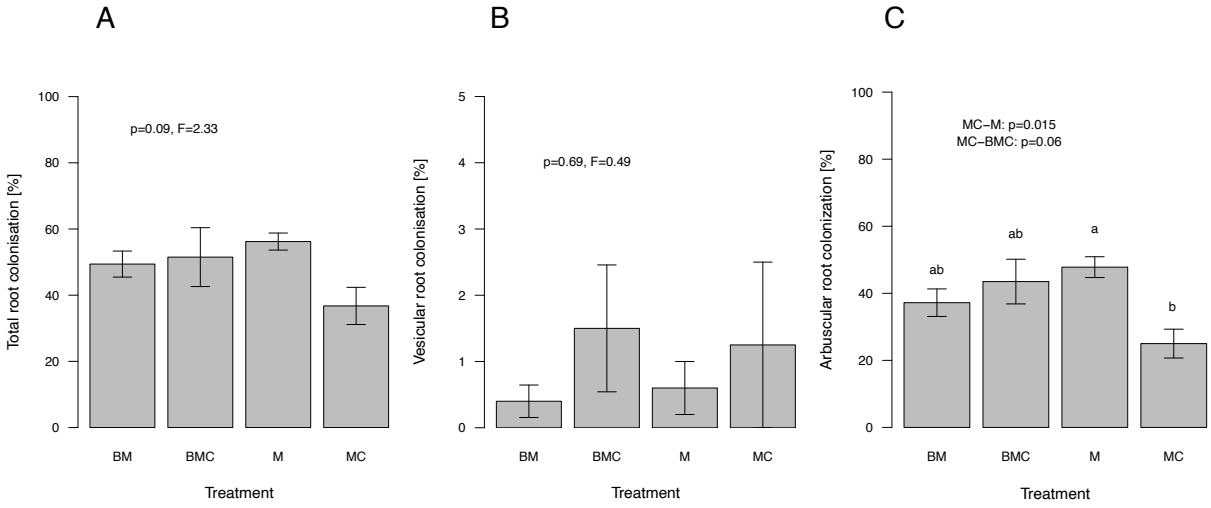

Figure 10: In celery root a significant increase in (C) arbuscular root colonization (p= 0.015) was detected in plots inoculated with *R. irregulare* strain SAF22 (M) compared to control plots (MC). Different letters show significant mean differences (p<0.05) according to Tukey test. Bars represent means of 6 replicated plots per treatment +/- 1 SE.

3.3 Effect on nutrient uptake

In the field trial with root parsley (E2), a significantly higher uptake of the nutrients N, C, Mg, P and Ca was observed in plants treated with *R. irregulare* compared to the control (Fig. 11). The calculation of the mycorrhizal uptake response (MUR) of the nutrients showed an increase in the concentration of P in the plant of 20 % (p= 0.009). The uptake of N was also increased by 19% and was significantly different from the control (p= 0.001). Carbon uptake was significantly increased by 20% by inoculation with *R. irregulare*l (p=0.001). Finally, a significant increase of 20% and 17% in the nutrient concentrations of Mg and Ca was observed treated with *R. irregulare* compared to the control (p=0.001, p= 0.011). For the nutrients potassium and sodium, no significant nutrient uptake response was observed (Table 3 in appendix B.1).

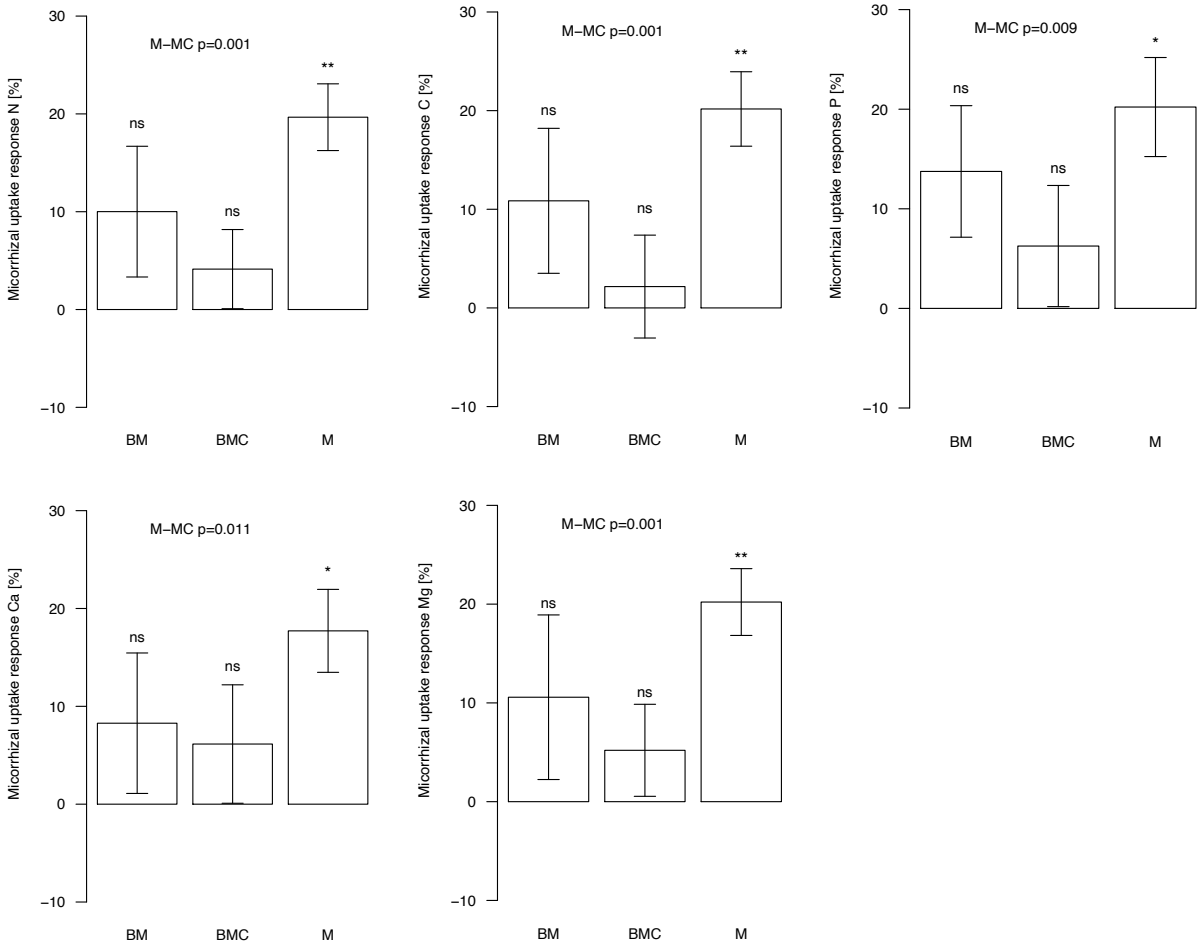

Figure 11: Mycorrhizal uptake response showing the significant percentage change of nutrient concentrations of N, C, P, Ca und Mg in root parsley roots inoculated with *R. irregulare*. Differences from the mean value of control treatments were assessed by one-sided t-tests. Test results are given above bars as ns > 0.1, * p < 0.05 and ** p < 0.001. Bars represent means of 6 replicated plots per treatment (five replicated plots for treatment BM) +/- 1 SE.

3.4 Effect on resistance to diseases

In the first field trial with celery (E1), only a mild infestation of the leaves with powdery mildew was detected at harvest. Since, in consultation with the farmer, this did not result in any crop losses, a disease assessment was not carried out in trial E1. In E2, a mild-moderate infestation of powdery mildew was assessed on the leaves at harvest. However, no significant difference in leaf infestation severity was detected between treatments (p=0.98)(Fig. 19 in appendix B.3).

The root health score results in root parsley were best for the control (MC) with the least fungal infestation on the root. The treatment with Bacillus (BMC) showed the highest root infestation with a significant percentage of yield loss (p=0.05) (Fig. 12A). For farmers, it may be of interest that the inoculation of *R. irregulare* results in the harvest of the highest amount of healthy plants (p=0.087) (Fig. 12B).

E2 A B

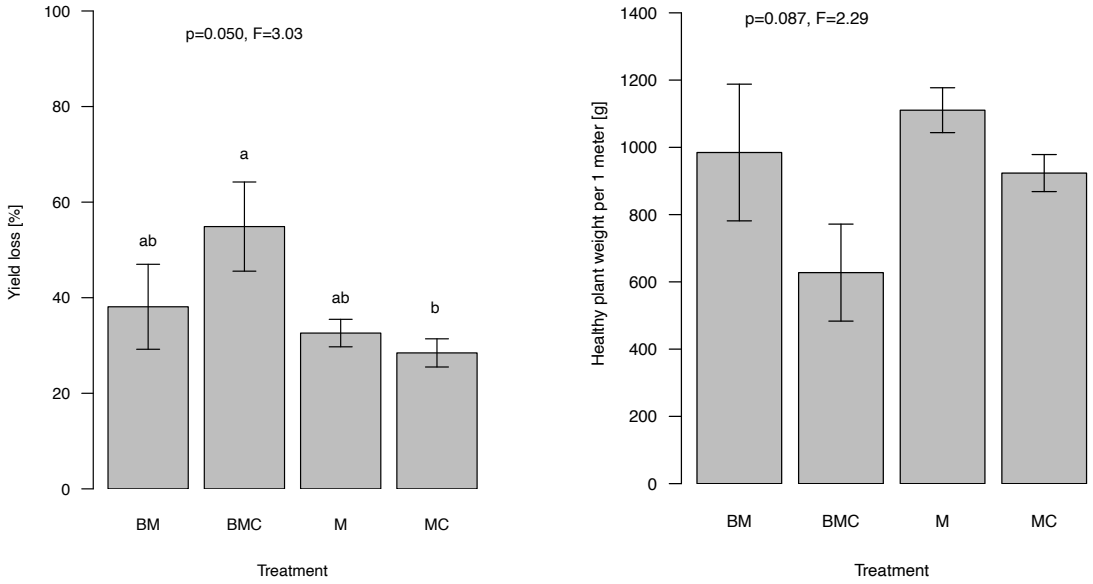

Figure 12: No significant effect of treatments M, BMC and BM on root health of root parsley was detected. (A) shows yield loss due to disease incidence on roots in root parsley. Roots in plots treated with Bacillus showed a significant yield loss. The yield loss shows the % weight of infected plants out of the healthy plant weight for each treatment. (B) Weight of healthy roots harvested along 1 meter was highest in treatment with R. irregulare, but not significant. Bars represent means of 6 replicated plots per treatment (five replicated plots for treatment BM) +/- 1 SE.

In celery root (E3), disease pressure from the leaf spot disease *Septoria apiicola* was already very high at the time of the first disease assessment, one month before harvest. The two plant health assessments showed a heavy leaf infestation by Septoria. In the second sampling, in addition to the leaf spot infestation, a yield loss due to *Pectobacterium carotovorum subsp. carotovorum* (Erwinia root rot) was detected in the roots. The purpose of the sampling was to determine the effect of the treatments on plant health by measuring plant height and disease

severity. However no correlation between plant height and treatment was found. Disease severity scales of the two assesments in celery root show lower leaf infestation by Septoria within the two treatments M and BM, but no significant difference from the other treatments (Fig. 13 and 14).

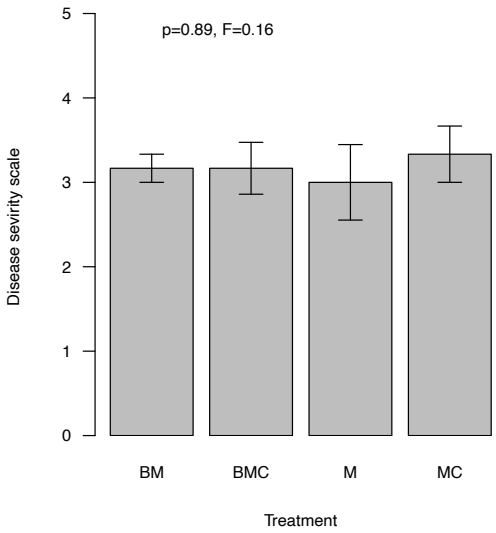

Figure 13: In the first scoring of celery root (E3) none of the treatments had a positive impact on disease suppression. On a scale with scores from 1= without disease to 5= only heartleaves not infected, plants within all Treatments were affected rather strongly by disease with a median score of 3 (majority of leaves infected) (p=0.81). Bars represent means of 4/5 replicated plots per treatment (five replicated plots for treatment BM) +/- 1 SE.

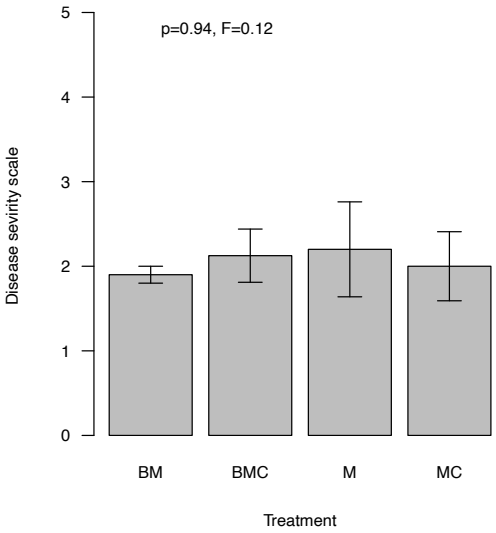

Figure 14: In the second scoring of disease impact in celery root (E3) there was no significant difference in plant height and disease severity between the treatments (p= 0.94). On a scale with scores from 1= only heartleaves not infected to 5= without disease, plants within all treatments were affected rather strongly by disease with a median score of 3 (majority of leaves dead). Bars represent means of 4/5 replicated plots per treatment (five replicated plots for treatment BM) +/- 1 SE.

4 Discussion

The aim of this work was to find out whether the well-characterized and efficiently colonizing AM fungus *R. irregulare* can establish in the root of celery, celery root and root parsley crops and can increase crop yield under field conditions, In addition the study investigated if the combination with *B. amyloliquefaciens* has additional positive effects on plant growth and health. In the following, the results of the three field trials are discussed with reference to literature. The first chapter discusses the establishment success of *R. irregulare* in the roots. This is followed by the evaluation of the effect of single and combined inoculation of the two biostimulants on plant growth and nutrient uptake. The third chapter is devoted to the effect on plant health and followed by a reflection of limitations of the study. An outlook on the implications in practice concludes the work.

4.1 Establishment of the inoculated fungus

The examination of root colonization demonstrated a symbiosis of both celery and root parsley with AM fungi. In all experiments, high colonization levels by AMF were detected in both inoculated and control plants. Treatments with *B. amyloliquefaciens* showed no significant effect on root colonization. Arbuscular root colonization was significantly increased by *R. irregulare* in celery root (E3). At the same time, celery root showed the lowest root colonization by native AM fungi in control plants. In contrast, the other two experiments showed higher root colonization levels by native AM fungi with a percentage of root length colonized of approximately 60% in control plants. Thus, the inoculated fungus could establish better in the experiment with the lowest root colonization by native AM fungi. These results confirm the findings of earlier studies, concluding that the establishment of inoculated AMF is dependent on abundance of native AMF in the soil. A lower abundance of native AMF communities in the soil may favor the establishment of inoculated AMF because more niche space is available for inoculated AMF (Niwa et al. 2018; Bender et al. 2019). In the field trials with root parsley (E2) and celery (E1), only a slight increase in total root colonization of inoculated plants was observed.

The highest total root colonization levels were found in celery plants inoculated with *R. irregulare* followed by root parsley and celery root. Despite the positive effect of *R. irregulare* inoculation on biomass in E2, no significant increase in root colonization compared to control could be detected in this experiment. Therefore, the effect on biomass can not be confirmed with the results of root colonization. Bender et al. (2019) however, demonstrated that the quantitative real time polymerase reaction (qPCR) method can detect an increase of the inoculated AMF in the plant root without this being reflected in an increase in total root

colonization levels. This could be based on the fact that inoculated AMF take the place of native AMF in the root and replace them. Schlaeppi et al. (2016) demonstrated that inoculation of *R. irregulare* can replace native AM fungal strains in plant roots. However, this assumption raises concerns about the inoculation of AMF as they may behave invasively and thus interfere with native AMF communities.

In this study, the highest plant available soil P levels were detected in field E3 (39 mg/kg) compared to E1 (27.95 mg/kg) and E2 (7.5 mg/kg). A significant increase in arbuscular root colonization by the inoculated fungus was observed in E3 with the highest P-level. Since only three fields were studied in this work, correlations between soil P levels and root colonization were not performed. However, research of Bender et al. (2019) confirms that establishment of inserted AMF can positively correlate with soil P values. The findings of Hamel et al. (1997) underline this cognizance by showing that high levels of soil P can negatively affect and decimate native AMF communities. This in turn creates more available niche space for introduced AMF.

4.2 Effect of *R. irregulare* and *B. amyloliquefaciens* on plant growth

The experiments show varying results on the influence of the AM fungus *R. irregulare* on plant growth of celery and root parsley under field conditions. A significant increase of biomass of 31% in root parsley was detected. The results of the mycorrhizal growth response confirmed these results with a MGR of 23% in root parsley. The influence of *R. irregulare* was also demonstrated in the nutrient uptake with a significant mycorrhizal uptake response of the nutrients N, P, C, Mg, and Ca in inoculated plants. In contrary, no correlation of biomass with root colonization was found.

The biomass results in the two celery experiments were not significant, with 5% increase in E1 and 1% increase in E3. However, it must be considered that in E1 the celery seedlings were inoculated too late, after the plants had already rooted in the soil. This stress factor could have negatively influenced the impact of inoculation. In In E3, some of the experimental plants were harvested by the farmer before testing could take place, thus reducing the number of replicates. In addition, the celery root plants in E3 were exposed to a high stress level caused by diseases, which led to a high failure rate of the plants and therefore fewer plants could be evaluated in some plots. In order to make a more precise statement on the positive influence of AMF on the celery crop, the experiments would have to be repeated with a higher number of replicates.

The results of the three field trials suggest that a positive influence on plant growth caused by inoculation with *R. irregulare* may depend on the crop variety and also on field specific factors. Kokkoris et al. (2019) confirmed the influence of field-specific factors on the establishment of AMF in several field trials. Different compositions of site-specific soil microbial communities may explain the high variation of the effect of AMF inoculations on plant growth in the field (Bender et al. 2019). In this regard, the influence of field-specific conditions such as microbial communities on the establishment of *R. irregulare* represents an exciting area of future research. In experiment E2, a tendency of a positive influence of the combined inoculation with Bacillus and AMF compared to the control was observed. However, the yields of the combined inoculation never exceeded the yields of the single inoculation of *R. irregulare*. It remains to be validated whether the colonization by *R. irregulare* is particularly facilitated if applied in combination with the bacteria *B. amyloliquefaciens*.

It is also interesting to note that the effect of *R. irregulare* on plant growth of root parsley was strongest even though the lowest amount of AMF inoculum was applied per plant in this experiment. This effect could be caused by the fact that root parsley was sown directly into the inoculum in the field. Thus, seedlings were already under the influence of the AMF inoculum before germination and during seedling development. This could have caused an additional positive influence on the establishment of the inoculated fungus in the plant root. This also marked the difference to the two trials with celery, where the inoculum was applied after planting the seedlings in the field soil, i.e. at a later stage of development than with root parsley. Studies by Mäder et al. (2005) and Douds et al. (2008) showed that seedlings inoculated with AMF before planting had higher yields than control plants. These results indicate that the timing of inoculation may play a greater role than the amount of inoculum applied. The influence of the timing of inoculation will need to be investigated in further trials.

4.3 Effects of *R. irregulare* and *B. amyloliquefaciens* on plant health

The disease infestation monitoring in the field trials E2 and E3 did not show any significant influence of inoculated AMF and Bacillus on the resistance to the diseases powdery mildew and Septoria. In E3, the reason for the relatively large variance of data within the treatments could be due to the fact that disease infestation by Septoria occurred very non-homogeneously in the field, a typical disease pattern for this disease. In E3, the celery root seedlings were planted directly neighboring an older set of celery root, which showed infestation by Septoria towards the end of the cultivation period. This greatly increased the risk of infection of the experimental plants and exposed the celery root plants to high disease pressure from Septoria early on. The fungus then spread in a circular and non-homogeneous manner from the

neighboring field into the experimental field (Fig. 15). Since the experimental plots with the different treatments were all affected by Septoria to different degrees of severity and for different durations, the disease assessments carried out are not very reliable with regard to the influence of AMF on plant health.

Figure 15: The spread of *Septoria apiicola* in celery root showed the typical herd-like disease pattern. The front rows of the trial were more severely affected by the disease than the back rows. Anne-Miamed Fehr

Also in root parsley no positive influence could be detected by inoculation with *R. irregulare*. However, the disease infestation does not necessarily have to be considered as a yield decimator. The fungal infestation on root parsley roots could be removed with some pressure when washing the roots. Although this could lead to increased expenses in the processing of the vegetables.

4.4 Limitations of the study

Since three different crops were investigated in this work, it was not possible to perform a meaningful comparison between the three field trials. A limitation of this bachelor thesis was also the detection methods of the inoculated biostimulants. On the one hand, the colonization by AMF in the root was not assessed with molecular methods on a species level. This made it difficult to draw reliable conclusions about the establishment success of the inoculated AM fungus. On the other hand, no investigations were carried out on the colonization of the root by *B. amyloliquefaciens*. Therefore, the influence of *B. amyloliquefaciens* could only be determined by plant weight. In addition, native microbial communities in the soil and their influence on the inoculated fungus and Bacteria were not investigated in this work, as this would exceed the framework of this bachelor thesis.

4.5 Outlook and implementation in praxis

Promoting AMF in the soil could be profitable for vegetable farms. The trial results show that AMF has the potential to increase yields. In severely degraded soils, inoculation may provide a solution strategy to reestablish AMF in the soil (Asmelash et al. 2016). Similarly, inoculation of AMF can be integrated into crop rotations by adding them after crops that do not form symbiotic relationships with AMF (e.g., crucifers) (Berset et al. 2011).

To promote native AMF preferably, soils are inoculated with native mycorrhizal fungi from the farms own field. In a study of Pellegrino et al. (2011) the results on plant productivity and quality improved with the application of native AMF compared to exotic AMF inoculants. Douds et al. (2010) have developed a method for propagating native AMF on the own farm. In the future, it will be important to develop further strategies to facilitate AMF application in the field.

In this trial, it was shown that a larger amount of inoculum does not necessarily lead to better results. In other words, the best results in biomass were obtained in the experiment with the smallest amount of inoculum applied per plant. The required amount of inoculum could be further investigated and suitable methods for application could be developed, such as seed coating. At the present time, application in seedling cultivation of vegetables in the greenhouse, before outplanting in the field could be easier than later application in the field. For field inoculation, strategies still need to be developed for adding the inoculum mechanically whilst sowing or planting. In seedling cultivation, the application of the inoculum would be easier because the inoculated substrate can be mixed easily with the seedling substrate. However, it should be noted that the inoculum must not influence the substrate too extensively in its properties such as water retention capacity and air permeability, in order to exclude negative effects of the Inoculum. In vegetable farms with their own seedling cultivation, the inoculum could be produced on the farm and added to the substrate for seedling cultivation (Douds et al. 2010).

The importance of the promotion of native AMF should not be neglected when considering to use the benefits of these fungi. For example, constant root penetration and low tillage intensity of the soil, avoidance of pesticides and the most diverse crop rotation possible are viable strategies for enhancing root colonization by native AMF (Mäder et al. 2000; Berset et al. 2011; Köhl et al. 2014; Bowles et al. 2017; Helander et al. 2018; Zhang et al. 2020). Through such measures, internal regulatory ecosystem functions can be promoted and AMF can thrive in the soil for longer periods of time and thus provide the desired benefits (Zhang et al. 2020).

5 Conclusion

In this bachelor thesis, high root colonization rates in celery, celery root and root parsley as well as a positive effect of *R. irregulare* on plant growth of root parsley could be demonstrated. This contributes to the broadening of knowledge on the ecological importance of AMF for crops in agricultural systems.

The effects of the two biostimulants have previously been studied under controlled and/ or sterile conditions in the laboratory or greenhouse. In the field, inoculated organisms are exposed to a variety of environmental factors. The effects of the two biostimulants on plant growth and health are therefore highly dependent on the specific conditions of the three field trial sites. The influence of field-specific conditions such as microbial communities on the establishment of *R. irregulare* represents an interesting area for future research.

To achieve more statistical power, it would be interesting to repeat this type of field trial with a larger number of replicates and at multiple sites including conventionally managed fields. Since no significant results were obtained in this trial with *B. amyloliquefaciens* as MHB for AMF, the co-inoculation of Bacillus, as well as other MHB with AMF under field conditions, should be further investigated. In addition to the effect on plant growth and health, it would be interesting to investigate whether and to what extent the introduced bacteria can establish themselves in the diverse microbial communities in field soils. Co-inoculations with microorganisms derived from natural soil communities or the rhizosphere of host plants could also present a strategy to enhance the success rate of AMF inoculations.

Arbuscular mycorrhizal fungi play an important role in the development of sustainable agricultural practices for the future. This study has been able to demonstrate the positive influence of AMF on plant growth and nutrient uptake under field conditions. A more profound understanding of the environmental factors influencing AMF as well as the functioning of the symbiosis between soil microorganisms and AMF and the identification of MHB, together may provide important guidelines for the development of new management methods in agriculture. This could contribute to the development of solution strategies to ensure nutrient sufficiency in extensive soil management. Thereby, sustainable agricultural cycles can be created, which are less dependent on fertilizer input and in which microbial communities can regulate themselves independently in the soil, whilst being additionally productive (Köhl et al. 2014; Zhang et al. 2020).

6 Literature

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7 Appendix

Table of contents:

A Experimental designs

A.1 Experimental design E1

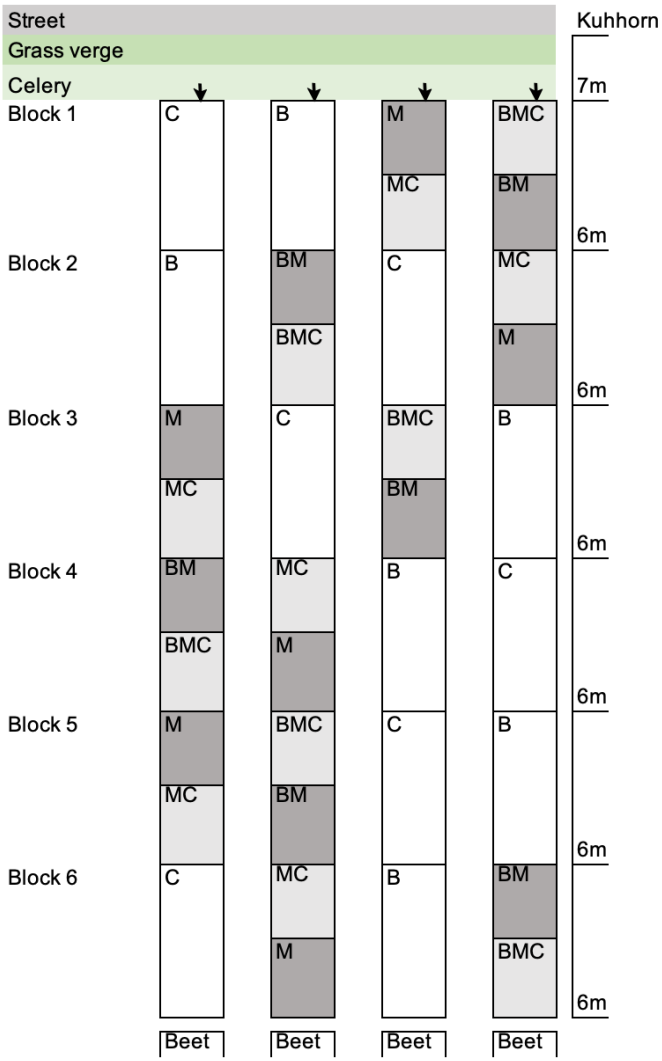

Figure 16: In the first experiment the treatments of M-MC and BM-BMC were paired.

A.2: Experimental design E3

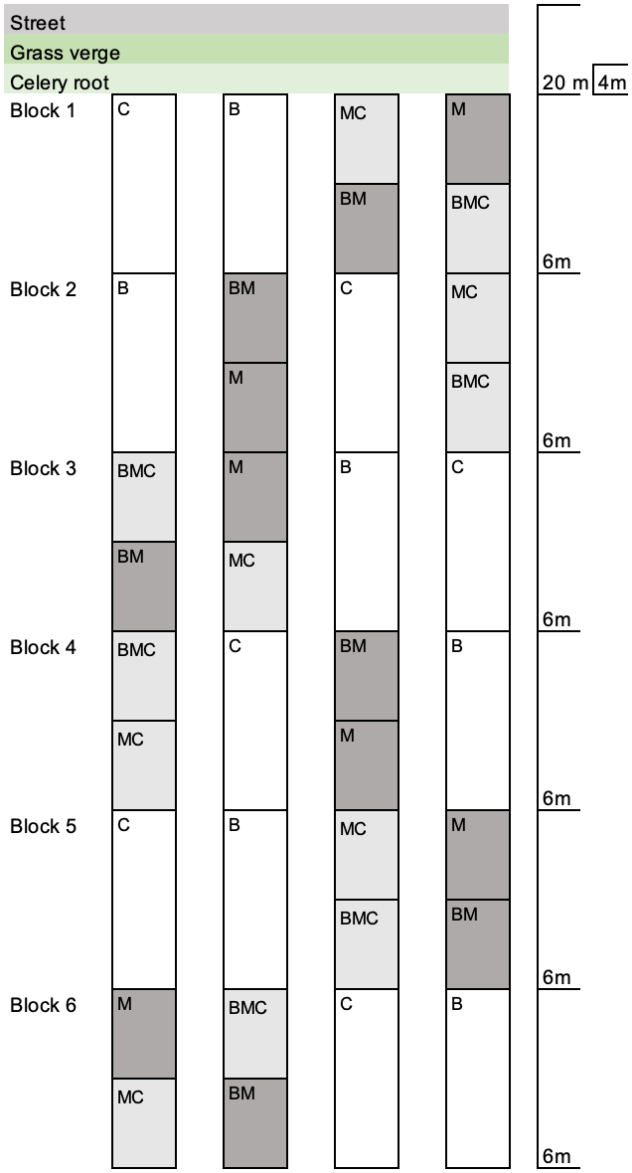

Figure 17: The set up oft he Field trial in celery root. After the first experiment the design was adapted and completely randomized in comparison to the first experiment in celery. The mycorrhizal plots and non-mycorrhizal plots without the fungus are no longer paired.

B Results

B.1: Table 3: Mean values of the calculated uptake reactions by mycorrhiza (MUR) and p-values derived from one sided t-tests are given for the analyzed nutrient concentrations in the field trial E2.

	MUR N [%]	p-value T-test	MUR P [%]	p-value T-test	MUR C [%]	p-value T-test	MUR K [%]	p-value T-test	MUR Ca [%]	p-value T-test	MUR Mg [%]	p-value T-test	MUR Na [%]	p-value T-test
M	**19.7**	**0.001**	**20.2**	**0.009**	**20.2**	**0.001**	13.8	0.27	17.7	**0.011**	**20.2**	**0.001**	12.3	0.063
BM	10.0	0.109	13.8	0.065	10.9	0.109	15.7	0.77	8.3	0.167	10.6	0.139	10.7	0.177
BMC	4.1	0.212	6.3	0.207	2.2	0.346	8.0	0.3	6.1	0.2	5.2	0.187	-1.4	0.534
MC	-0.376		-0.846		-0.47		-1.3		-0.999		-0.347		-0.7	

B.2: Fresh and dry root weight per plant in E2

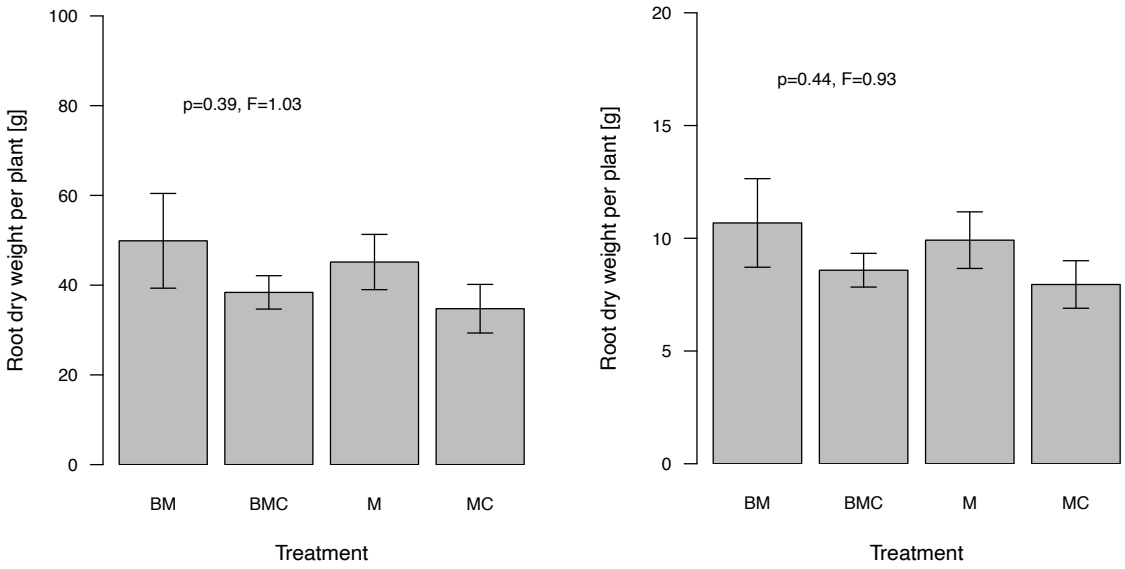

Figure 18: No significant difference in fresh or dry weight per plant was detected in root parsley. Bars represent means of 6 replicated plots per treatment (five replicated plots for treatment BM) +/- 1 SE.

B.3: Bonitur Field E2

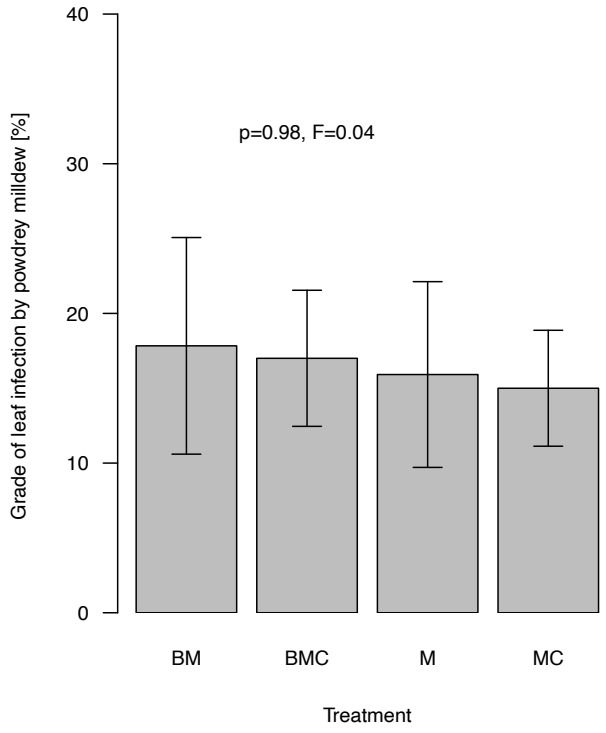

Figure 19: No significant difference in the grade of leaf infection by powdrey mildew was detected in root parsley. Bars represent means of 6 replicated plots per treatment (five replicated plots for treatment BM) +/- 1 SE.

C Declaration of independence

Zürcher Hochschule für Angewandte Wissenschaften

zh aw Life Sciences und Facility Management

Erklärung betreffend das selbständige Verfassen einer Bachelorarbeit im Departement Life Sciences und Facility Management

Mit der Abgabe dieser Bachelorarbeit versichert der/die Studierende, dass er/sie die Arbeit selbständig und ohne fremde Hilfe verfasst hat.

Der/die unterzeichnende Studierende erklärt, dass alle verwendeten Quellen (auch Internetseiten) im Text oder Anhang korrekt ausgewiesen sind, d.h. dass die Bachelorarbeit keine Plagiate enthält, also keine Teile, die teilweise oder vollständig aus einem fremden Text oder einer fremden Arbeit unter Vorgabe der eigenen Urheberschaft bzw. ohne Quellenangabe übernommen worden sind.

Bei Verfehlungen aller Art treten Paragraph 39 und Paragraph 40 der Rahmenprüfungsordnung für die Bachelor- und Masterstudiengänge an der Zürcher Hochschule für Angewandte Wissenschaften vom 29. Januar 2008 sowie die Bestimmungen der Disziplinarmassnahmen der Hochschulordnung in Kraft.

Ort, Datum: Unterschrift:

Zürich, 12.01.2021 [redacted]

Das Original dieses Formulars ist bei der ZHAW-Version aller abgegebenen Bachelorarbeiten im Anhang mit Original-Unterschriften und -Datum (keine Kopie) einzufügen.

Zürcher Fachhochschule

D Article

Der Gemüsebau/Le Maraîcher
Einfluss von Mykorrhiza auf das Wachstum von Gemüsekulturen

Mehr Gemüseertrag durch Mykorrhiza

Feldversuche in Sellerie und Wurzelpetersilie zeigen: Die Förderung von Mykorrhiza pilzen im Boden eröffnet neue Ansätze,um die Nährstoffaufnahme von Gemüse zu erhöhen und gleichzeitig den Düngereinsatz zu reduzieren.

Was sind Mykorrhizapilze?
Mykorrhizapilze kommen weltweit im Boden vor. Sie leben in einer symbiontischen Beziehung mit einer Vielzahl an Landpflanzen. Dazu gehören auch die meisten landwirtschaftlichen Kulturen. Mit ihrem feinen Netz aus Pilzfäden erreichen Mykorrhizapilze Nährstoffe im Boden, welche für die Wurzeln der Pflanzen ohne sie unerreichbar bleiben. Über bäumchen-förmige Strukturen in den Zellen der Pflanzenwurzel tauschen die Mykorrhizapilze diese Nährstoffe gegen Kohlenstoff aus der Photosynthese der Wirt-Pflanze. Für die landwirtschaftliche Produktion stellt sich die Frage, wie sich dieser Nährstoffaustausch auf das Wachstum von Kulturpflanzen auswirkt.

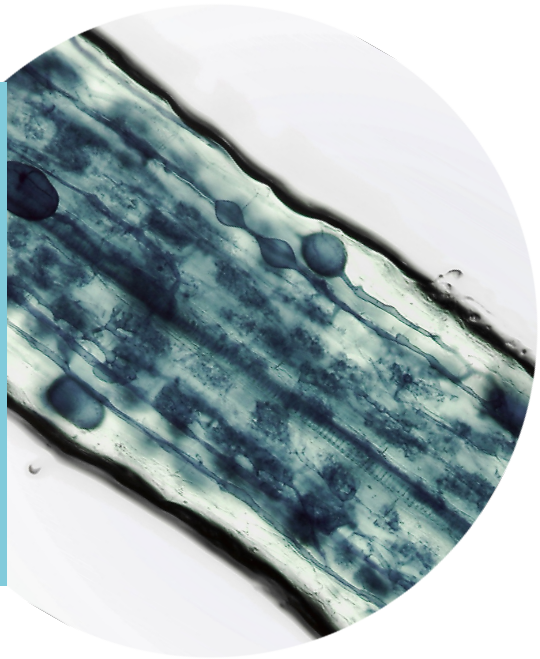

Abb. 1 Mykorrhizapilze in einer Wurzel von Wurzelpetersilie (ungeimpfte Pflanze). Anne-Miamed Fehr

Feldversuche in Wurzelpetersilie und Sellerie

In drei Feldversuchen, durchgeführt von Agroscope in Zusammenarbeit mit dem Bildungs- und Beratungszentrum (BBZ) Arenenberg und der Zürcher Fachhochschule für Angewandte Wissenschaften (ZHAW), wurde untersucht, ob die Zugabe eines Mykorrhizapilzes das Pflanzenwachstum positiv beeinflussen kann. Teil der Studie waren ein Demeter und zwei biologische Gemüsebaubetriebe im Kanton Thurgau. Die Kulturen Stangensellerie, Wurzelpetersilie und Knollensellerie wurden mit dem natürlichen

Mykorrhizapilz der Art *R. irregulare* vor der Aussaat bzw. bei der Pflanzung geimpft (Abb.2).
Am Ende der Kulturdauer wurde das Produktgewicht und die Besiedlung der Wurzel durch Mykorrhiza untersucht.

Abb. 2: Links: Feldsaat Wurzelpetersilie in geimpften Boden, rechts Impfung bei der Pflanzung von Stangensellerie. Anne-Miamed Fehr

Die Kulturen zeigen hohe Besiedlung durch Mykorrhiza

Alle drei Kulturen zeigten sowohl in den beimpften als auch in Pflanzen ohne Impfung eine hohe Besiedlung durch Mykorrhizapilze in ihren Wurzeln (Abb.1). Daraus lässt sich schliessen, dass in den untersuchten Böden einheimische Mykorrhiza bereits zahlreich vorhanden waren. Geimpfte Pflanzen zeigten jedoch tendenziell eine höhere Besiedlung durch Mykorrhiza. Dies verdeutlicht, dass sich Mykorrhilzapilze erfolgreich in den Wurzeln von Knollen- und Stangensellerie, sowie Wurzelpetersilie etablieren können. Der Einfluss der Impfung mit dem Mykorrhizapilz wurde in der Kultur Wurzelpetersilie, mit einem Ertragsanstieg von 31% am stärksten sichtbar gefolgt von Knollensellerie mit 5% Ertragssteigerung (Abb.3).

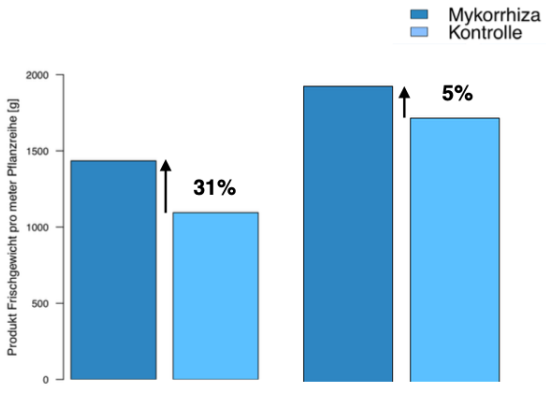

Abb.3: In Wurzelpetersilie konnte der Ertrag mit Mykorrhiza um 31% gesteigert werden.

Umsetzung in die Praxis

Die Versuchsergebnisse zeigen, dass Mykorrhiza potential hat die Erträge zu steigern. In stark degenerierten Böden kann die Impfung eine Lösungsstrategie darstellen, um Mykorrhiza neu im Boden anzusiedeln. Ebenso kann eine Impfung von Mykorrhizapilzen in die Fruchtfolge integriert werden, indem sie nach Kulturen, die keine Symbiose mit Mykorrhiza eingehen (z.B. Kreuzblütler), hinzugefügt werden. Vorzugsweise werden die Böden mit einheimischen Mykorrhizapilzen vom eigenen Feld geimpft. Douds et al. (2010) haben eine Methode zur Vermehrung von Mykorrhizapilzen auf dem eigenen Betrieb entwickelt. In Zukunft ist es wichtig Strategien zu entwickeln, um die Anwendung im Feld zu vereinfachen. Hierzu startet in diesem Jahr ein neues, von der Gebert Rüf Stiftung gefördertes, Forschungsprojekt von Agroscope und dem BBZ Arenenberg.

Auch ohne Impfung können die natürlich im Boden vorkommenden Mykorrhizapilze gefördert werden. So sind eine konstante Durchwurzelung und eine geringe Bearbeitungsintensität des Bodens, der Verzicht auf Pestizide und eine möglichst diverse Fruchtfolge förderlich. Durch solche Massnahmen können Mykorrhizapilze längerfristig im Boden bestehen und die gewünschten Vorteile erbringen.

Weiterführende Quellen

Douds DD (2010) How to Innoculate Arbuscular Mycorrhizal Fungi on the Farm, Part 1. In: Rodale Institute. https://rodaleinstitute.org/science/articles/how-to-innoculate-arbuscular-mycorrhizal-fungi-on-the-farm-part-1/.

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